HYDROPHOBICITY OF PHENYLALANINE 1586 WITHIN THE PORE OF THE HUMAN NAV1.4 SODIUM CHANNEL IS A DETERMINANT OF LOCAL ANESTHETIC BINDING

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The molecular receptor for local anesthetic drugs (LA), such as mexiletine, lies within the pore of voltage-gated sodium channels (NaCh). In particular two aromatic aminoacids of the 6th segment of NaCh domain IV, Tyr1593 and Phe1586 in hNav1.4, may allow the formation of hydrophobic and π-cation interactions with the aromatic moiety and the tertiary amine of mexiletine (1). In a previous study, we showed that the β-adrenoceptor drugs, clenbuterol and propanolol, directly block NaChs in a manner redundant to LA (2). In contrast, salbutamol and nadolol did not affect NaChs, probably because of the presence of two hydroxyl groups on the aromatic moiety of these drugs, which may impede interaction with the LA binding site. In the present study, we verified this hypothesis by testing mexiletine derivatives with hydroxyl groups on the aryloxy moiety on hNa$_V$1.4 channels expressed in tsA201 cells. The block of NaChs by the drugs was determined by measuring the reduction of sodium currents elicited from the holding potential of -120 mV to -30 mV at 0.1 Hz. The concentration-response curves were fitted with a first-order binding function. The half-maximum inhibitory concentration (IC_{50}) was 248 µM for mexiletine, 1089 µM for para-hydroxy-mexiletine (p-OH-M), 1990 µM for 2'-hydroxy-mexiletine (2'-OH-M), and 10480 µM for 2',6'-dihydroxy-mexiletine (2',6'-$(OH)_2$-M). The IC_{50} was well correlated with the drug hydrophobicity expressed as logP value. To gain further insight, the F1586C mutation was engineered into the wild-type hNav1.4 template, allowing the replacement of the hydrophobic phenylalanine, part of the LA receptor, by an hydrophilic cysteine residue. The IC_{50} for C1586 mutant block was 1340 µM for mexiletine, 1132 µM for p-OH-M, 2994 µM for 2'-OH-M, and 1342 µM for 2',6'-$(OH)_2$-M. Thus the mutation strongly affects mexiletine block as expected from the contribution of F1536 to the LA binding site. In contrast, the two monohydroxyl derivatives were quite insensitive to the mutation, suggesting that F1536 is no more involved in the binding of these drugs to NaCh. In addition, the C1536 mutant was far more sensitive to 2',6'-$(OH)_2$-M than the wild-type F1536, suggesting that the mutation creates a new, favorable ambient for binding of the less hydrophobic derivative. Supported by Telethon grant GGP04140.

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